

University of Dundee

## Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2

Kotelevtsev, Yuri; Brown, R W; Fleming, S; Kenyon, Christopher; Edwards, Christopher R. W.; Seckl, Jonathan R.

*Published in:*  
Journal of Clinical Investigation

*DOI:*  
[10.1172/JCI4445](https://doi.org/10.1172/JCI4445)

*Publication date:*  
1999

*Document Version*  
Publisher's PDF, also known as Version of record

[Link to publication in Discovery Research Portal](#)

*Citation for published version (APA):*  
Kotelevtsev, Y., Brown, R. W., Fleming, S., Kenyon, C., Edwards, C. R. W., Seckl, J. R., & Mullins, J. J. (1999). Hypertension in mice lacking 11beta-hydroxysteroid dehydrogenase type 2. *Journal of Clinical Investigation*, 103(5), 683-689. <https://doi.org/10.1172/JCI4445>

### General rights

Copyright and moral rights for the publications made accessible in Discovery Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from Discovery Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain.
- You may freely distribute the URL identifying the publication in the public portal.

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

# Hypertension in mice lacking 11 $\beta$ -hydroxysteroid dehydrogenase type 2

Yuri Kotelevtsev,<sup>1</sup> Roger W. Brown,<sup>2</sup> Stewart Fleming,<sup>3</sup> Christopher Kenyon,<sup>2</sup> Christopher R.W. Edwards,<sup>2</sup> Jonathan R. Seckl,<sup>2</sup> and John J. Mullins<sup>1</sup>

<sup>1</sup>Centre for Genome Research, University of Edinburgh, Edinburgh EH9 3JQ, Scotland, United Kingdom

<sup>2</sup>Molecular Endocrinology Laboratory, Molecular Medicine Centre, University of Edinburgh, Western General Hospital, Edinburgh EH4 2XU, Scotland, United Kingdom

<sup>3</sup>Department of Pathology, Teviot Place, Edinburgh EH8 9AG, Scotland, United Kingdom

Address correspondence to: Yuri Kotelevtsev, Centre for Genome Research, University of Edinburgh, Kings Buildings, West Mains Road, Edinburgh EH9 3JQ, Scotland, United Kingdom. Phone: 44-131-650-58-70; Fax: 44-131-667-01-64; E-mail: Yuri.Kotelevtsev@ed.ac.uk

Received for publication June 30, 1998, and accepted in revised form January 18, 1999.

Deficiency of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2) in humans leads to the syndrome of apparent mineralocorticoid excess (SAME), in which cortisol illicitly occupies mineralocorticoid receptors, causing sodium retention, hypokalemia, and hypertension. However, the disorder is usually incompletely corrected by suppression of cortisol, suggesting additional and irreversible changes, perhaps in the kidney. To examine this further, we produced mice with targeted disruption of the 11 $\beta$ -HSD2 gene. Homozygous mutant mice (11 $\beta$ -HSD2<sup>-/-</sup>) appear normal at birth, but ~50% show motor weakness and die within 48 hours. Both male and female survivors are fertile but exhibit hypokalemia, hypotonic polyuria, and apparent mineralocorticoid activity of corticosterone. Young adult 11 $\beta$ -HSD2<sup>-/-</sup> mice are markedly hypertensive, with a mean arterial blood pressure of 146  $\pm$  2 mmHg, compared with 121  $\pm$  2 mmHg in wild-type controls and 114  $\pm$  4 mmHg in heterozygotes. The epithelium of the distal tubule of the nephron shows striking hypertrophy and hyperplasia. These histological changes do not readily reverse with mineralocorticoid receptor antagonism in adulthood. Thus, 11 $\beta$ -HSD2<sup>-/-</sup> mice demonstrate the major features of SAME, providing a unique rodent model to study the molecular mechanisms of kidney resetting leading to hypertension.

*J. Clin. Invest.* 103:683–689 (1999)

## Introduction

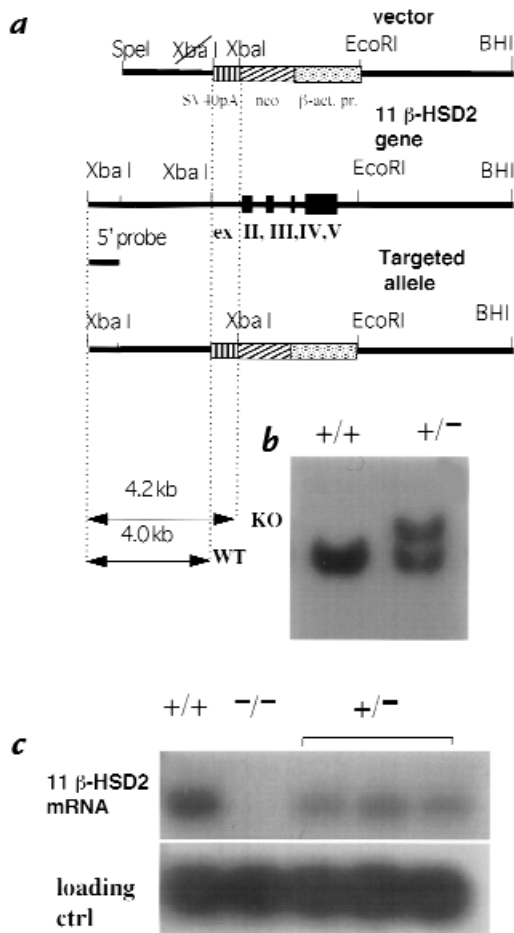
Sustained alterations of blood pressure require resetting of renal mechanisms maintaining salt–water balance (1). The latter is critically dependent on sodium resorption in the distal tubule of the nephron, which is regulated by mineralocorticoid hormones (principally aldosterone). Indeed, most human monogenic hypertensive and hypotensive syndromes reflect mutations in genes that determine electrolyte transport in the distal portion of the nephron, either directly (2, 3) or through alterations in mineralocorticoid- or glucocorticoid-signaling pathways (4, 5).

Although physiological glucocorticoids (corticosterone in rats and mice, cortisol in humans) bind to purified mineralocorticoid receptors (MR) with affinities similar to aldosterone *in vitro* (6), *in vivo* MR are selectively activated by aldosterone. This selectivity is produced by 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ -HSD2), which catalyzes the rapid metabolism of glucocorticoids to inert 11-keto forms (11-dehydrocorticosterone, cortisone) (7). In the syndrome of apparent mineralocorticoid excess (SAME) (8), deficiency of renal 11 $\beta$ -HSD2 allows physiological glucocorticoids (cortisol, corticosterone) to illicitly activate MR in the distal tubule, producing sodium retention, severe hypertension, and hypokalemia (7, 9, 10). How-

ever, the disorder is usually incompletely corrected by suppression of cortisol, suggesting that irreversible changes also occur, possibly in the kidney (11). To examine the pathophysiological basis for this critical hypertensive condition further, we produced mice with targeted disruption of the 11 $\beta$ -HSD2 gene.

## Methods

**Construction of the replacement vector and gene targeting.** Two overlapping genomic fragments, encompassing 10.5 kb of the 5' region, the complete 11 $\beta$ -HSD2 gene, and 9 kb of the 3' downstream region, were cloned from a 129 Sv library in the  $\lambda$ PS vector (12). Sequencing of all five exons showed a complete match with mouse 11 $\beta$ -HSD2 cDNA (13). A replacement vector was constructed in pBS- $\beta$ KnpA, containing the neomycin resistance gene flanked by the human  $\beta$ -actin promoter and a SV40 polyadenylation signal sequence. A 5-kb *EcoRI*–*Bam*HI fragment placed immediately downstream of exon 5 was used as a 3' homology arm. The 2.7-kb *SpeI*–*XbaI* fragment from intron A (5' arm of homology) was subcloned into the *SpeI* site of pBS- $\beta$ KnpA, so that the 3' *XbaI* site was not recreated after ligation. Replacement vector linearized with *SpeI* and *SalI* was electroporated into embryonic stem (ES) cells (line E-14), and G418-resistant clones were selected as described (14). Two hundred fifty neomycin-resistant clones were analyzed by Southern blotting, using *XbaI* analytical restriction digestion and hybridization with a 0.8-kb *XbaI*–*SpeI* 5' external probe. Clones with spe-



**Figure 1**

Targeted inactivation of 11β-HSD2 gene. (a) Structure of the targeting vector, the 11β-HSD2 locus, and the targeted allele. (b) Southern blot hybridization analysis of *Xba*I-digested genomic DNA from neomycin-resistant nontargeted (+/+) and targeted (+/-) ES clones. (c) Northern blot analysis of 11β-HSD2 expression. RNA (10 μg) from kidney of wild-type (+/+), homozygous (-/-) mutants and three heterozygous (+/-) littermates was hybridized with mouse 11β-HSD2 and control (*ctrl*) U-1 snRNA cDNA probes. 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; ES, embryonic stem cells.

cific recombination events were selected, and recombination at the 3' end was confirmed by restriction digestion with *Ssp*I and *Not*I and subsequent hybridization with the *Ssp*I-*Bam*HI 3' external probe (not shown). Cells from four targeted ES cell clones were injected into C57BL/6 blastocysts and transferred into C57BL/6 × CBA foster mothers. Chimeras were bred to MF1 females and the progeny genotyped by Southern blot analysis of DNA digested with *Bam*HI and *Sac*I. Northern blot analysis was performed as described (15).

**Assays of 11β-HSD activities.** Homogenates from placentas at embryonic day 18.5 (E18.5) were assayed for 11β-HSD2, a nicotinamide adenine dinucleotide (NAD)-dependent exclusive 11β-dehydrogenase, and for the lower-affinity 11β-HSD1 isozyme, which, *in vitro*, is a bidirectional reduced nicotinamide adenine dinuc 11β-HSD2 bioassays were carried out using placental tissue homogenates (0.25 mg protein/ml) incubated at 37°C with 400 μM NAD and 12 nM [<sup>3</sup>H]corticosterone (16). 11β-HSD1 was assayed in the reductase direction using 400 μM NADPH and 12 nM [<sup>3</sup>H]-11-dehydrocorticosterone. Steroids were extracted and quantified by HPLC (17).

Plasma renin activity was measured using the established assay (18). Aldosterone levels were measured by using a radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, California, USA).

**Mineralocorticoid actions of glucocorticoids *in vivo*.** Adult male wild-type and 11β-HSD2-null mice were injected with vehicle (10% ethanol/saline) subcutaneously for 3 days, followed by dexamethasone (100 μg/kg) to suppress endogenous corticosterone for 3 days, and finally dexamethasone (100 μg/kg) and replacement corticosterone (10 mg/kg) for 3 days. Urine was collected in metabolism cages for 24 h beginning 4 h after the start of each treatment, and electrolytes were measured by automatic analyzer. Basal 24-h urine collections and blood samples from chronically cannulated animals were also obtained for electrolyte estimations.

**Blood pressure measurement.** Blood pressures were measured in 3-month-old chronically cannulated, conscious males and females as described previously (19). Microcatheters (Braintree Scientific Inc., Braintree, Massachusetts, USA) were microsurgically implanted into the abdominal aorta. Blood pressures were recorded directly from conscious, freely moving mice for 10-min periods between 10 and 11 am on 3–5 consecutive days from 48 h after the operations.

**Histological preparations.** Kidneys were fixed in 10% buffered formalin and embedded in paraffin wax, and multiple adjacent 4-μm sections were cut and mounted on glass slides. After dehydration, the sections were stained with hematoxylin and eosin, periodic acid-Schiff, and Martius scarlet blue. Sections were examined by an experienced pathologist (S. Fleming), who was blinded to the genotype. Glomeruli and tubule segments were identified by positional and morphological criteria, as described previously (20), because immunocytochemistry-based identification of tubule types is unreliable in circumstances of tubule hypertrophy and hyperplasia (21, 22). Individual distal tubule diameters were measured by image analysis using a Zeiss laser scanning microscope (14). Twenty tubule diameters were measured in each of three -/- and wild-type animals at 3 months of age.

Tissue for electron microscopy was immersion-fixed as 1-mm cubes in 4% glutaraldehyde in cacodylate buffer. The tissue blocks were postfixed in osmium tetroxide, washed in cacodylate buffer, and dehydrated through graded alcohols. Blocks were embedded in araldite resin. Ultra-thin sections were cut, mounted on copper grids, and stained with uranyl acetate and lead citrate. They were examined on a Phillips CM 12 transmission electron microscope.

**Table 1**

Concentration of electrolytes in 11β-HSD2<sup>-/-</sup> homozygous mutants, 11β-HSD2<sup>+/-</sup> heterozygous, and 11β-HSD2<sup>+/+</sup> wild-type adult mice

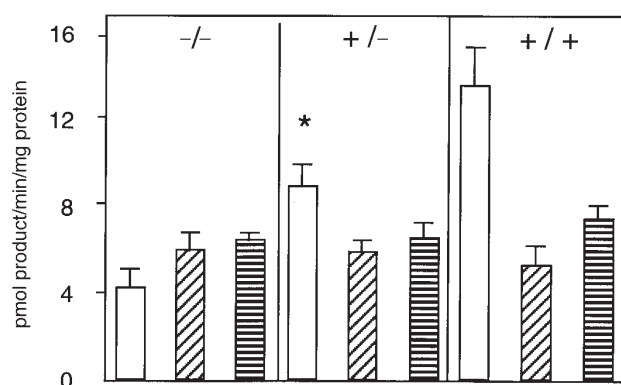
	11β-HSD2 <sup>-/-</sup> n = 4	11β-HSD2 <sup>+/-</sup> n = 4	11β-HSD2 <sup>+/+</sup> n = 4
Plasma sodium (mmol/l)	152.1 ± 1.35	149.5 ± 0.56	150.3 ± 0.84
Plasma potassium (mmol/l)	3.2 ± 0.31 <sup>A,D</sup>	4.9 ± 0.27	4.74 ± 0.2
Plasma chloride (mmol/l)	109.3 ± 1.75 <sup>B,C</sup>	117.2 ± 0.86	114.7 ± 1.67
Plasma creatinine (μmol/l)	19.1 ± 0.9	20.1 ± 0.74	19.8 ± 0.54
Plasma sodium/potassium	50.2 ± 4.60 <sup>A,D</sup>	31.1 ± 1.59	32.1 ± 1.46

The concentration of electrolytes in 11β-HSD2<sup>-/-</sup> homozygous mutants, 11β-HSD2<sup>+/-</sup> heterozygous, and 11β-HSD2<sup>+/+</sup> wild-type adult mice was measured in plasma separated from terminal cardiac blood samples of 3-month-old mice (mean ± SE). Variance was assessed by ANOVA followed by the Newman-Keuls *post hoc* test.

<sup>A</sup>P < 0.001, <sup>B</sup>P < 0.05 compared with 11β-HSD2<sup>-/-</sup>.

<sup>C</sup>P < 0.05, <sup>D</sup>P < 0.001 compared with 11β-HSD2<sup>+/+</sup>.

11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2.



**Figure 2**

Variation in 11 $\beta$ -HSD2 dehydrogenase and 11 $\beta$ -HSD1 oxidoreductase activities in placentas with different 11 $\beta$ -HSD2 genotypes. Activities (mean  $\pm$  SEM) expressed as conversion of nmol [ $^3$ H]-steroid substrate (either corticosterone for 11 $\beta$ -dehydrogenase or 11-dehydrocorticosterone for 11 $\beta$ -reductase) to pmol product/mg protein/min. (Open bars) NAD-dependent 11 $\beta$ -corticosterone dehydrogenase activity. (Diagonally striped bars) NADP-dependent 11 $\beta$ -corticosterone dehydrogenase activity. (Horizontally striped bars) NADPH-dependent 11-dehydrocorticosterone reductase activity. Note that activity due to 11 $\beta$ -HSD1 does not vary with genotype. In contrast, NAD-dependent predominantly 11 $\beta$ -HSD2 activity varies with genotype, resulting in overall NADP-preferring 11 $\beta$ -HSD activity in 11 $\beta$ -HSD2 $^{-/-}$  mice. The residual NAD-dependent activity in 11 $\beta$ -HSD2 $^{-/-}$  placentas presumably reflects activity of 11 $\beta$ -HSD1 enzyme. \*Significantly greater than corresponding 11 $\beta$ -HSD2 $^{-/-}$  value ( $P < 0.001$ ) and significantly smaller than corresponding wild-type (+/+) values ( $P < 0.001$ ). NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate.

To determine whether changes in renal structure were readily reversible, 3-month-old 11 $\beta$ -HSD2 $^{-/-}$  mice were injected subcutaneously once a day with the mineralocorticoid receptor antagonist spironolactone (10 mg/kg, in vegetable oil) for 14 days and then sacrificed. Tissue was collected for histology.

**Statistics.** Data were assessed by ANOVA followed by the Newman-Keuls *post hoc* test or Student's *t* tests, as appropriate. Plasma renin activity data were not normally distributed and were analyzed by the Mann-Whitney test. Significance was set at  $P < 0.05$ . Values are means  $\pm$  SEM.

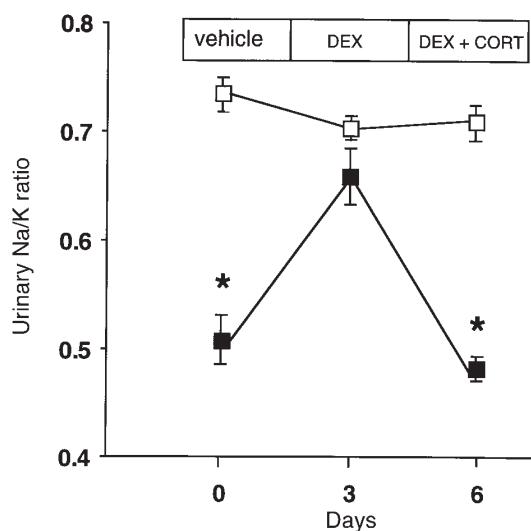
## Results

A null mutation of the 11 $\beta$ -HSD2 locus was generated by replacing the genomic fragment encompassing exons 2–5 with a neomycin-resistance cassette (Fig. 1*a*) through homologous recombination in mouse 129 ES cells. Specific recombination was detected in 12 of 250 G418-resistant colonies by Southern blot analysis with 5' (Fig. 1*b*) and 3' (not shown) external probes. Three targeted clones injected into blastocysts gave rise to chimeras capable of germline transmission. Three independent 11 $\beta$ -HSD2 $^{-/-}$  transgenic lines were established by crossing chimeras to MF1 outbred females and subsequent intercross breeding of heterozygotes.

Genotyping of 191 intercross progeny at weaning (21 days) showed clear deviation from mendelian ratios (31  $-/-$ , 113  $+/-$ , 47  $+/+$ ;  $P < 0.01$ ), with less than half of the expected number of 11 $\beta$ -HSD2 $^{-/-}$  mice found. In contrast, in fetuses at E18.5 ratios were close to mendelian, with 12 of 45 (26.7%) of the 11 $\beta$ -HSD2 $^{-/-}$  genotype. 11 $\beta$ -HSD2 $^{-/-}$  mice appeared normal at birth, but within 48 hours

showed ~50% mortality. Neonatal death was usually preceded by motor weakness and reduced suckling and accompanied by abdominal swelling with intestinal loop dilatation. All 11 $\beta$ -HSD2 $^{-/-}$  mice surviving >48 hours after birth reached adulthood. No 11 $\beta$ -HSD2 mRNA was detected in 11 $\beta$ -HSD2 $^{-/-}$  mouse kidney, whereas heterozygotes showed a 50% reduction of 11 $\beta$ -HSD2 mRNA expression (Fig. 1*c*). Subsequently, 16% of 11 $\beta$ -HSD2 males and 13% (of 75 total) females died suddenly between two and four months of age (one 11 $\beta$ -HSD2 $^{-/-}$  female died suddenly four hours postpartum, and two died toward the end of lactation of their first litters). In most cases ( $n = 6$  males and 6 females) postmortem analysis revealed no obvious cause of death (such as stroke or myocardial infarction/rupture), though one female died with a ruptured aorta and pericardial bleeding, and another had hemorrhaged around a kidney capsule. Both male and female 11 $\beta$ -HSD2 $^{-/-}$  mice surviving until adulthood were fertile and gave rise to live homozygous offspring.

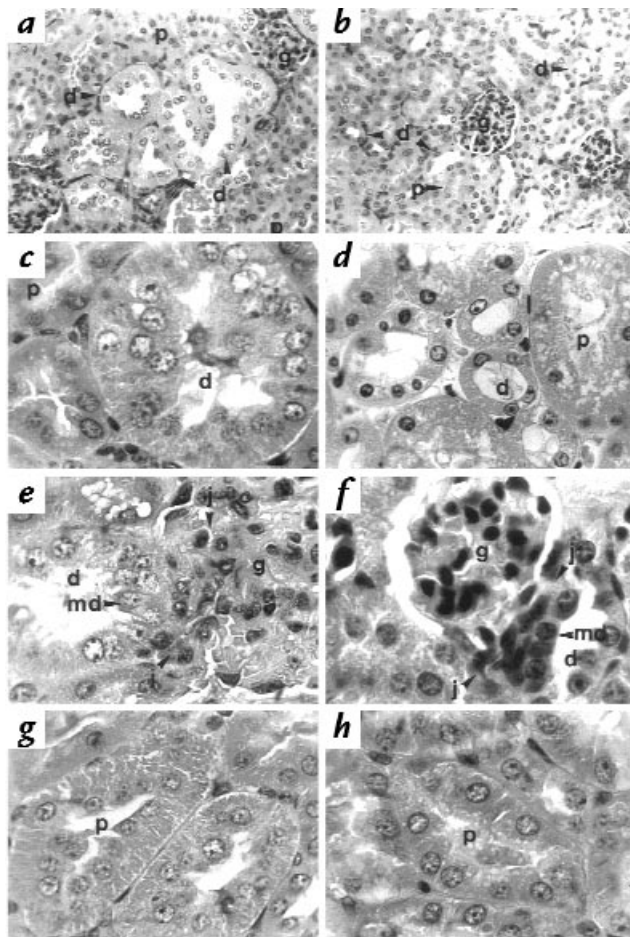
Placental NAD-dependent 11 $\beta$ -dehydrogenase activity at E18.5 (full gestation at E19.5) was  $13.7 \pm 1.9$  pmol/min/mg protein ( $45.5 \pm 6.4\%$  conversion) of corticosterone to inert 11-dehydrocorticosterone in wild-type (+/+)  $9.0 \pm 0.7$  pmol/min/mg protein ( $30.1 \pm 2.4\%$  conversion) in heterozygous (+/-) and  $4.3 \pm 0.6$  pmol/min/mg protein ( $14.4 \pm 2.1\%$ ) in homozygous mutant 11 $\beta$ -HSD2 $^{-/-}$  mice (Fig. 2). Mouse placentas at E18.5 also contain 11 $\beta$ -HSD type 1, an NADPH-dependent enzyme that acts as an oxidoreductase (regenerating active glucocorticoids) in intact cells (23). Mice homozygous for targeted disruption of the 11 $\beta$ -HSD1 gene do not show alterations in plasma



**Figure 3**

Corticosterone acts as a mineralocorticoid in 11 $\beta$ -HSD2 $^{-/-}$  mice. Wild-type (open squares;  $n = 4$ ) and 11 $\beta$ -HSD2 $^{-/-}$  (closed squares;  $n = 4$ ) mice were injected with vehicle (days -3 to 0), followed by dexamethasone (DEX; 100  $\mu$ g/kg, days 0–3) to suppress endogenous corticosterone, and finally dexamethasone with replacement corticosterone (DEX + CORT; 10 mg/kg, days 3–6). Urine was collected for the last 24 h of each treatment and electrolytes measured by automated analyzer. The abnormally low urinary Na/K ratio in 11 $\beta$ -HSD2 $^{-/-}$  mice is reversed by dexamethasone and recurs with corticosterone replacement, whereas the treatments have no effect in wild-type mice. \*Significantly different from corresponding wild-type value ( $P < 0.0005$ ).





**Figure 4**

Hyperplasia and hypertrophy of the distal renal tubular epithelium in  $11\beta$ -HSD2<sup>-/-</sup> mice. (a) In adult  $11\beta$ -HSD2<sup>-/-</sup> mice, the distal tubules (d) show considerable enlargement: the tubular diameter is increased threefold compared with the distal tubule in the wild-type mice (b). Note that the proximal tubules (p) and glomeruli (g) are unaffected in the  $11\beta$ -HSD2<sup>-/-</sup> mice. (c) Hyperplasia is apparent in the  $11\beta$ -HSD2<sup>-/-</sup> mouse distal tubule (d), which shows over 20 cells in the cross-sectional profile compared with (d) the four to six cells observed in controls. (e) The distal tubular enlargement (d) is evident in  $11\beta$ -HSD2<sup>-/-</sup> mice at the anatomical origin of the distal tubule at the juxtaglomerular apparatus (j) adjacent to the glomerulus (g), compared with (f) the nonenlarged wild-type distal tubule. md, macula densa. (g) Proximal tubule (p) is normal in  $11\beta$ -HSD2<sup>-/-</sup> kidneys.  $11\beta$ -HSD2<sup>-/-</sup> proximal tubules are indistinguishable from (h) wild-type proximal tubules. The tubules are of similar diameter and cell number in cross section. They are lined by cuboidal cells with granular cytoplasm and a brush border in both animals. The same magnification was used in  $11\beta$ -HSD2<sup>-/-</sup> and corresponding wild-type control photographs. In a and b, H&E  $\times 60$ ; in c and d, H&E  $\times 120$ ; in e–h, H&E  $\times 180$ . H&E, hematoxylin and eosin.

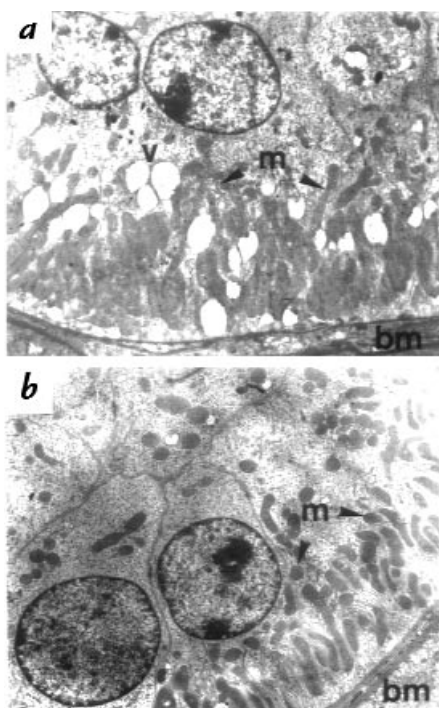
electrolytes or blood pressure (24).  $11\beta$ -HSD1 activity was equal in placentas of all three genotypes (Fig. 2). No differences in fetal or placental weights were found at E18.5 (fetus: homozygous mutant  $-/-$   $784 \pm 16$  mg,  $n = 11$ ; heterozygous  $+/-$   $797 \pm 20$  mg,  $n = 19$ ; wild-type  $+/+$   $790 \pm 34$  mg,  $n = 5$ ; placenta:  $-/-$   $142 \pm 9.4$  mg;  $+/-$   $147 \pm 7.2$  mg;  $+/+$   $152 \pm 14$  mg), nor were there weight differences between genotypes (either sex) at weaning.

Adult male and female  $11\beta$ -HSD2<sup>-/-</sup> mice showed hypotonic polyuria (24-h urine volume  $3.2 \pm 0.3$  ml vs.  $2.1 \pm 0.2$  ml;  $P < 0.005$ ), with reduced urinary concentrations of sodium, potassium, and creatinine ( $61 \pm 10\%$ ,  $75 \pm 9\%$ , and  $71 \pm 5\%$  of wild-type, respectively;  $P < 0.001$ ).  $11\beta$ -HSD2<sup>-/-</sup> null mice had normal plasma sodium and creatinine but showed marked hypokalemia and hypochloremia (Table 1), with plasma potassium levels as low as  $2.4 \pm 0.2$  mmol/l in chronically catheterized males and  $2.7 \pm 0.2$  mmol/l in females ( $n = 3$ ). Plasma electrolytes in heterozygotes did not differ significantly from wild-type. Eight  $11\beta$ -HSD2<sup>-/-</sup> mice had fully suppressed plasma renin activity below the level of detection of angiotensin I (AI) ( $<0.05$  ng AI/mg protein/min), whereas levels in wild-type mouse plasma were significantly higher ( $0.75 \pm 0.40$  ng AI/mg/min;  $P < 0.05$ ,  $n = 8$ ). Plasma aldosterone levels were profoundly suppressed in  $11\beta$ -HSD2<sup>-/-</sup> male and female mice ( $232 \pm 93$  pmol/l,  $n = 6$ ) compared with wild-type controls ( $3085 \pm 397$  pmol/l,  $n = 6$ ;  $P < 0.0001$ ).

To determine whether endogenous glucocorticoids were responsible for the apparent mineralocorticoid excess, we examined the effects on urinary Na/K ratios of suppression of corticosterone by dexamethasone and subsequent corticosterone replacement. Dexamethasone reversed the low Na/K ratio in  $11\beta$ -HSD2<sup>-/-</sup> mice but had no effect in wild-type controls (Fig. 3). Replacing corticosterone in dexamethasone-treated  $11\beta$ -HSD2<sup>-/-</sup> mice recreated low urinary Na/K ratios, whereas controls were again unaffected.

To examine whether  $11\beta$ -HSD2 gene disruption and the consequent apparent mineralocorticoid excess caused hypertension, blood pressures were measured directly in three-month-old conscious male and female mice using chronic abdominal aortic cannulae as described previously (19).  $11\beta$ -HSD2<sup>-/-</sup> mice were hypertensive, with mean blood pressures of  $146 \pm 2$  mmHg in males ( $n = 6$ ) and  $155 \pm 4$  mmHg in females ( $n = 3$ ), compared with  $121 \pm 2$  mmHg in wild-type males ( $n = 4$ ,  $P < 0.001$ ) and  $122 \pm 3$  mmHg in wild-type females ( $n = 5$ ,  $P < 0.05$ ). Male heterozygotes had similar blood pressures ( $114 \pm 4$  mmHg,  $n = 5$ ) to wild-type controls. Although the three-month-old  $11\beta$ -HSD2<sup>-/-</sup> mice tended to exhibit an increased heart weight/body weight ratio compared with age-, sex-, and weight-matched wild-type controls ( $-/-$   $6.8 \pm 0.7$  mg/g,  $n = 6$ ;  $+/-$   $5.6 \pm 0.4$  mg/g,  $n = 9$ ;  $P < 0.15$ ), the difference was not statistically significant. Histological examination of the hearts of  $11\beta$ -HSD2<sup>-/-</sup> mice ( $n = 6$ ; 3 males and 3 females) showed no evidence of interstitial fibrosis, but there were clear morphological features of cardiac myocyte hypertrophy (not shown).

Three-month-old  $11\beta$ -HSD2<sup>-/-</sup> mice exhibited marked renal enlargement ( $353 \pm 13$  mg,  $n = 6$ ; vs.  $277 \pm 14$  mg,  $n = 9$ ;  $P = 0.003$  in the wild-type). Histology revealed that this increase in renal mass was the consequence of distal tubular enlargement. The glomeruli, proximal tubules, medullary rays, medulla, and collecting ducts were all normal using the light (Fig. 4a) and electron microscopes (not shown). However, the distal tubules showed a two- to fourfold increase in diameter ( $11\beta$ -HSD2<sup>-/-</sup> mean distal tubular diameter  $61.3$   $\mu$ m, range  $42.6$ – $96.8$   $\mu$ m;  $11\beta$ -HSD2<sup>+/+</sup>



**Figure 5**

Electron microscopy of the distal tubular epithelial cells of 11 $\beta$ -HSD2<sup>-/-</sup> (a) and wild-type controls (b). Distal tubular epithelial cells from 11 $\beta$ -HSD2<sup>-/-</sup> animals show an increase in size. The subnuclear, or basal, pole is expanded with apical displacement of the nucleus. The number of mitochondria (m) in each distal tubular cell is increased, although the mitochondria remain located at the expanded basal pole (bm, basal membrane). Occasional membrane-enclosed vacuoles (v) are seen in the cytoplasm of the distal tubular epithelial cells from the 11 $\beta$ -HSD2<sup>-/-</sup> animals.  $\times 3,800$ .

mean distal tubular diameter 25.7  $\mu$ m, range 21.2–28.3  $\mu$ m) as a result of both hyperplasia and hypertrophy of the epithelium (Fig. 4a and c). These changes were seen at the origin of the distal tubule at the juxtaglomerular apparatus and in the convoluted portion (Fig. 4e). The hypertrophy resulted in increased cell size, with apical displacement of nuclei and increased granularity. Electron microscopy revealed clear differences between the 11 $\beta$ -HSD2<sup>-/-</sup> animals and the wild-type controls (Fig. 5, a and b). The distal tubular epithelial cells from 11 $\beta$ -HSD2<sup>-/-</sup> animals showed an increase in size, with the subnuclear, (basal) appearing expanded with apical displacement of the nucleus. The 11 $\beta$ -HSD2<sup>-/-</sup> animals showed an increase in the number of mitochondria in each distal tubular cell, although these remained located at the expanded basal pole. Occasional membrane-bound enclosed vacuoles were seen in the cytoplasm of the distal tubular epithelial cells from the 11 $\beta$ -HSD2<sup>-/-</sup> animals. All described morphological tubular changes were identical in male and female mice.

The kidneys of gestational day 18.5 and newborn animals were histologically normal. At one week old, two out of three of the 11 $\beta$ -HSD2<sup>-/-</sup> animals showed a minor degree of tubular dilatation, without hyperplasia or hypertrophy, affecting <1% of tubular profiles. By three weeks old, hypertrophy and hyperplasia of the distal tubular epithelium was evident in all of the 11 $\beta$ -HSD2<sup>-/-</sup> kidneys examined.

Treatment of adult 11 $\beta$ -HSD2<sup>-/-</sup> mice with spironolactone for 14 days improved the hypokalemia ( $5.5 \pm 0.6$  mmol/l) but had no effect upon the histological hyperplasia and hypertrophy of the distal tubule.

## Discussion

Targeted disruption of the 11 $\beta$ -HSD2 gene was successful, with no 11 $\beta$ -HSD2 mRNA detected in the kidneys of homozygous mutants and 50% reduction in heterozygotes. Measurement of 11 $\beta$ -dehydrogenase and 11 $\beta$ -reductase activities in the presence of different cofactors and substrates in placentas also supports the absence of 11 $\beta$ -HSD2 activity (a high-affinity NAD-dependent 11 $\beta$ -dehydrogenase) in homozygous mutants, with approximately 50% of wild-type activity in heterozygotes. Although the residual 11 $\beta$ -dehydrogenase activity with NAD in placentas of 11 $\beta$ -HSD2<sup>-/-</sup> fetuses raises the possibility of a novel 11 $\beta$ -HSD isoform, this seems more likely merely to reflect 11 $\beta$ -HSD1, which may continue moderate activity without added NADP, probably due to use of endogenous cosubstrate NADP, itself increased by exogenous NAD (17). 11 $\beta$ -HSD1 activity was unaffected by mutation of 11 $\beta$ -HSD2 in the placenta. The mutant phenotype appeared to be recessive, with detectable effects confined to homozygotes, and these findings were in accord with the lack of apparent disorders in parents or heterozygous sibs of children with SAME (25). However, heterozygous animals have only been examined up to four months of age, and effects later in life cannot be excluded.

Up to 50% of homozygous mice died within the first 48 hours after birth. Homozygous newborn pups in the intercross litters appeared weaker and less successful in competition for suckling. The abdominal swelling and intestinal dilatation observed in some 11 $\beta$ -HSD2<sup>-/-</sup> neonates before death suggests intestinal ileus. Other 11 $\beta$ -HSD2<sup>-/-</sup> pups died suddenly, possibly of cardiac arrest. Both processes are compatible with the effects of severe hypokalemia, clearly found in surviving adult 11 $\beta$ -HSD2<sup>-/-</sup> animals. The neonatal mortality mimics the documented perinatal and infant mortality of children with SAME (25, 26). Later deaths appeared either to reflect the hemorrhagic complications of severe hypertension or were unexplained and referred to possible cardiac events secondary to the electrolyte disturbances.

The most obvious phenotype of 11 $\beta$ -HSD2<sup>-/-</sup> mice was hypotonic polyuria, with 24-hour urine volumes increased by 50%. Hypokalemia causes polyuria due to nephrogenic diabetes insipidus and polydipsia in humans (27) and presumably underlies the polyuria in 11 $\beta$ -HSD2<sup>-/-</sup> mice. Hypokalemia appears secondary to urinary losses, driven by excessive mineralocorticoid action (low urinary Na/K ratio). In 11 $\beta$ -HSD2<sup>-/-</sup> mice, endogenous and exogenous corticosterone shows illicit mineralocorticoid action, effects absent from wild-type littermates. The main result of activation of mineralocorticoid receptors in the distal tubule is enhanced sodium resorption by the epithelial cells through the epithelial Na<sup>+</sup> channel (28), which ultimately results in elevation of blood pressure (1). Indeed, male and female 11 $\beta$ -HSD2<sup>-/-</sup> animals have severe hypertension with mean blood pressures above 145 mmHg.



The most striking structural changes in 11 $\beta$ -HSD2<sup>-/-</sup> mice are in the hyperplasia and hypertrophy confined to the distal tubule, with the proximal tubules, loops of Henle, and collecting ducts appearing normal. This type of distal tubular pathology has not been reported in other polyuric or hypokalemic syndromes that result in marked cytoplasmic vacuolation affecting the proximal tubules and has minor changes only in the distal tubules with no evidence of hyperplasia or hypertrophy (29). The changes are, however, similar to the effects of chronic furosemide administration (20), in which increased distal tubular sodium transport occurs as a consequence of increased sodium delivery to the distal tubule after inhibition of inward salt transport by the loop diuretic. In 11 $\beta$ -HSD2-null mice, the pathology is presumably a consequence of increased mineralocorticoid activity. Hyperplasia of the distal tubular epithelium, with increased total mitochondria and basal-membrane surface area, would be predicted to be the consequence of the energy requirement of overstimulation of active ion transport by mineralocorticoid receptor activity. 11 $\beta$ -HSD2 and mineralocorticoid receptor mRNA are confined to the distal nephron (30). We are not aware of any studies of renal biopsies from patients with SAME to allow comparison with the mice.

Kidneys from E18.5 11 $\beta$ -HSD2<sup>-/-</sup> fetuses showed no morphological or histological abnormalities. High expression of mineralocorticoid receptor mRNA in the distal nephron develops between E18.5 and just after birth (at E19.5) (30). Thus, only around birth do distal tubule epithelia of 11 $\beta$ -HSD2<sup>-/-</sup> animals first become exposed to glucocorticoid overactivation of mineralocorticoid receptors, findings consistent with glucocorticoid mediation of the distal tubular pathology. Intriguingly, in SAME patients, hypertension is usually not fully reversed by cortisol suppression. The marked structural abnormalities found in the adult 11 $\beta$ -HSD2<sup>-/-</sup> mouse distal tubule and the lack of reversal of these changes with mineralocorticoid receptor antagonism in adult life may explain this and focuses attention on renal structure in patients with chronic steroid hypertension.

In contrast to the striking renal pathology, the observed cardiac myocyte hypertrophy would be compatible with a secondary effect of hypertension alone. The lack of cardiac fibrosis, which has been associated with conditions of mineralocorticoid excess (not apparent mineralocorticoid excess), conforms with suggestions that this effect is mediated by MR unprotected by 11 $\beta$ -HSD2 and therefore requires elevated plasma levels of corticosteroids.

The mammalian placenta (and the fetus until midgestation) highly expresses 11 $\beta$ -HSD2, which may exclude high levels of maternal glucocorticoids from fetal tissues during sensitive periods of prenatal growth and development. Indeed, in rats and humans, placental 11 $\beta$ -HSD activity near term correlates with birth weight (31, 32) and pharmacological inhibition of fetoplacental 11 $\beta$ -HSD reduces birth weight (33). The lack of birth-weight changes in 11 $\beta$ -HSD2<sup>-/-</sup> mice suggests either that there are compensatory developmental mechanisms or, more likely, that this species, which shows early midgestational loss of 11 $\beta$ -HSD2 gene expression in the placenta and fetal tissues

(30), is less dependent on 11 $\beta$ -HSD2-mediated exclusion of glucocorticoids for maintenance of birth weight, which predominantly reflects growth in late gestation.

The 11 $\beta$ -HSD2<sup>-/-</sup> mice not only model SAME and advance our current understanding of its etiology, but will also allow more general insights into disorders of real or apparent mineralocorticoid excess, such as Conn's and Liddle's syndromes and glucocorticoid-remediable hyperaldosteronism (34, 2, 5), in which there is poorly understood variation in the degree of hypokalemia, hypertension, and the response to treatment. The mice will be useful in screening for new pharmaceutical compounds alleviating volume-dependent hypertension and also in studies of basic molecular mechanisms of mineralocorticoid responses in normal and pathological states.

### Acknowledgments

We thank Andrew Smith for  $\lambda$ PS library; William Skarnes and Meng Li for pBS- $\beta$ KnpA vector; Jan Ure, Gillian Brooker, Morag Meikle, and David Fettes for skilful technical assistance; and Matthew Sharp for discussion. The work was supported by a Programme grant from the Wellcome Trust (to J.R. Seckl, C.R.W. Edwards, and J.J. Mullins), and by funding from the The Biotechnology and Biological Sciences Research Council, The Commission of the European Communities concerted action Transgeneur, and the High Blood Pressure Foundation. J.R. Seckl and J.J. Mullins are the recipients of a Wellcome Senior Research Fellowship and a Wellcome Principal Fellowship respectively. R.W. Brown is a Medical Research Council Clinician-Scientist Fellow.

- Guyton, A.C. 1991. Blood pressure control—special role of the kidneys and body fluids. *Science*. **252**:1813–1816.
- Shimkets, R.A., et al. 1994. Liddle's syndrome: heritable human hypertension caused by mutations in the beta subunit of the epithelial sodium channel. *Cell*. **79**:407–414.
- Simon, D.B., et al. 1997. Mutations in the chloride channel gene, CLC-NKB, cause Bartter's syndrome type III. *Nat. Genet.* **17**:171–178.
- White, P.C., Vitec, A., Dupont, B., and New, M.I. 1988. Characterization of frequent deletions causing steroid 21-hydroxylase deficiency. *Proc. Natl. Acad. Sci. USA*. **85**:4436–4440.
- Lifton, R.P., et al. 1992. A chimaeric 11 $\beta$ -hydroxylase/aldosterone synthase gene causes glucocorticoid-remediable aldosteronism and human hypertension. *Nature*. **355**:262–265.
- Arriza, J.L., et al. 1987. Cloning of human mineralocorticoid receptor complementary DNA — structural and functional kinship with the glucocorticoid receptor. *Science*. **237**:268–275.
- Stewart, P.M., et al. 1987. Mineralocorticoid activity of liquorice: 11 $\beta$ -hydroxysteroid dehydrogenase deficiency comes of age. *Lancet*. **2**:821–823.
- Ulick, S., et al. 1979. A syndrome of apparent mineralocorticoid excess associated with defects in the peripheral metabolism of cortisol. *J. Clin. Endocrinol. Metab.* **49**:757–764.
- Edwards, C.R.W., et al. 1988. Localisation of 11 $\beta$ -hydroxysteroid dehydrogenase — tissue specific protector of mineralocorticoid receptor. *Lancet*. **2**:986–989.
- Mune, T., Rogerson, F.M., Nikkila, H., Agarwal, A.K., and White, P.C. 1995. Human hypertension caused by mutations in the kidney isozyme of 11 beta-hydroxysteroid dehydrogenase. *Nat. Genet.* **10**:394–399.
- Stewart, P.M., Corrie, J.E.T., Shackleton, C.H.L., and Edwards, C.R.W. 1988. Syndrome of apparent mineralocorticoid excess: a defect in the cortisol-cortisone shuttle. *J. Clin. Invest.* **82**:340–349.
- Nehls, M., Messerle, M., Sirulnik, A., Smith, A.J.H., and Boehm, T. 1994. Two large insert vectors,  $\lambda$ PS and  $\lambda$ KO, facilitate rapid mapping and targeted disruption of mammalian genes. *Biotechniques*. **17**:770–775.
- Cole, T.J. 1995. Cloning of the mouse 11-beta-hydroxysteroid dehydrogenase type-2 gene — tissue-specific expression and localization in distal convoluted tubules and collecting ducts of the kidney. *Endocrinology*. **136**:4693–4696.
- Clark, A.F., et al. 1997. Renin-1 is essential for normal renal juxtaglomerular cell granulation and macula densa morphology. *J. Biol. Chem.* **272**:18185–18190.

15. Chomczynski, P., and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159.
16. Low, S.C., et al. 1993. Regulation of 11 $\beta$ -hydroxysteroid dehydrogenase by sex steroids in vivo: further evidence for the existence of a second dehydrogenase in rat kidney. *J. Endocrinol.* **139**:27–35.
17. Brown, R.W., Chapman, K.E., Edwards, C.R.W., and Seckl, J.R. 1993. Human placental 11 beta-hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform. *Endocrinology.* **132**:2614–2621.
18. Millar, J.A., Leckie, B.J., and Morton, J.J. 1980. A micro-assay for active and total renin concentration in human plasma based on antibody trapping. *Clin. Chim. Acta.* **101**:5–15.
19. Sharp, M.G.F., et al. 1996. Targeted inactivation of the Ren-2 gene in mice. *Hypertension.* **28**:1126–1131.
20. Kaissling, B., Bachmann, S., and Kriz, W. 1985. Structural adaptation of the distal convoluted to prolonged furosemide treatment. *Am. J. Physiol.* **248**:F374–F381.
21. Fleming, S., and Matthews, T.J. 1987. Renal tubular antigens in regenerative epithelium and renal carcinoma. *Br. J. Urol.* **60**:103–109.
22. Grone, H.J., Weber, K., Grone, E., Helmchen, U., and Osborn, M. 1987. Co-expression of keratin and vimentin in damaged and regenerating tubular epithelia of the kidney. *Am. J. Pathol.* **129**:1–8.
23. Jamieson, P.M., Chapman, K.E., Edwards, C.R.W., and Seckl, J.R. 1995. 11  $\beta$ -Hydroxysteroid dehydrogenase is an exclusive 11  $\beta$ -reductase in primary cultures of rat hepatocytes: effect of physicochemical and hormonal manipulations. *Endocrinology.* **136**:4754–4761.
24. Kotelevtsev, Y., et al. 1997. 11 $\beta$ -Hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid inducible responses and resist hyperglycaemia on obesity or stress. *Proc. Natl. Acad. Sci. USA.* **94**:14924–14929.
25. White, P.C., Mune, T., and Agarwal, A.K. 1997. 11 $\beta$ -Hydroxysteroid dehydrogenase and the syndrome of apparent mineralocorticoid excess. *Endocr. Rev.* **18**:135–156.
26. Krozowski, Z.S., Stewart, P.M., Obeyesekere, V.R., Li, K., and Ferrari, P. 1997. Mutations in the 11  $\beta$ -hydroxysteroid dehydrogenase type II enzyme associated with hypertension and possibly stillbirth. *Clin. Exp. Hypertens.* **19**:519–529.
27. Berl, T., Linas, S.L., Aisenbrey, G.A., and Anderson, R.J. 1977. On the mechanism of polyuria in potassium depletion. *J. Clin. Invest.* **60**:620–625.
28. Grunder, S., and Rossier, B.C. 1997. A reappraisal of aldosterone effects on the kidney: new insights provided by epithelial sodium channel cloning. *Curr. Opin. Nephrol. Hypertens.* **6**:35–39.
29. Cherg, J., Cotran, R.S., Sinniah, R., Sakaguchi, H., and Sobin, C.H. 1985. *Renal disease: classification of tubulo-interstitial diseases*. WHO. Igaku-Shoin Medical Publishers. Tokyo, Japan.
30. Brown, R.W., et al. 1996. The ontogeny of 11 $\beta$ -hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. *Endocrinology.* **137**:794–797.
31. Benediktsson, R., Lindsay, R., Noble, J., Seckl, J.R., and Edwards, C.R.W. 1993. Glucocorticoid exposure in utero: new model for adult hypertension. *Lancet.* **341**:339–341.
32. Stewart, P.M., Rogerson, F.M., and Mason, J.I. 1995. Type 2 11 $\beta$ -hydroxysteroid dehydrogenase messenger RNA and activity in human placenta and fetal membranes: its relationship to birth weight and putative role in fetal steroidogenesis. *J. Clin. Endocrinol. Metab.* **80**:885–890.
33. Lindsay, R.S., Lindsay, R.M., Edwards, C.R.W., and Seckl, J.R. 1996. Inhibition of 11 $\beta$ -hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. *Hypertension.* **27**:1200–1204.
34. Gordon, R.D. 1997. Primary aldosteronism: a new understanding. *Clin. Exp. Hypertens.* **19**:857–870.